

**AMENDMENTS TO THE TITLE**

Please amend the present title of this application to read:

**“ ANTIBODIES AGAINST S100A8 AND S100A9 PROTEINS FOR MODULATING  
INFLAMMATORY REACTIONS “**

## AMENDMENTS TO THE SPECIFICATION

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### DISCLOSURE OF THE INVENTION

One object of the present invention is to provide a method for systemic modulation of an inflammatory reaction in an individual, a human or an animal; in ~~need~~need, comprising administering to the individual an effective amount of A chemotactic factor inhibitor, the chemotactic factor being selected from the group consisting of an S100 protein, a protein of the MRP family, calprotectin, and calgranulin.

The modulation can totally or partially inhibit the inflammatory reaction or totally or partially increase the inflammatory reaction.

The inflammatory reaction may be selected from the group consisting of arthritis, chronic polyarthritis, rheumatoid arthritis, gout, asthma, psoriasis, paraneoplastic syndrome, tumor-induced inflammatory diseases, turbid effusions, collagenosis, postinfectious arthritis, seronegative spondylarthritis, vasculitis, sarcoidosis, arthrosis, cell chemotaxis, cell migration, cell recruitment, proteolysis, oxidative burst, and radical oxydation.

The cell that can be chemoattracted by the compound and method of the present invention can be selected from the group consisting of a neutrophil, a monocyte, a platelet, a synoviocyte, a macrophage, a lymphocyte, a leukocyte, and a phagocytic cell.

According to one object of the present invention, the administration can be performed by intravenous, oral, intranasal, subcutaneous, topical, or intraperitoneal administration.

The method of the present invention is preferably performed on an animal that is a mammal.

According to another object of the invention, an effective amount can be an amount of S100 protein inhibitor effective to induce inhibition or activation of an inflammatory reaction.

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Figs. 9A to 9C illustrate the presence of S100A9 and S100A8/A9 in the serum of mice injected with LPS;

Figs. 10A to 10C illustrate the accumulation of neutrophils in blood after i.v. injection of S100A8, S100A9, and S100A8/A9;

Figs. 11A to 11D illustrate the mobilization of neutrophils from the bone marrow to the blood after injection of S100A8 and S100A9; and

Fig 12 illustrates the effect of anti-S100A8 and anti-S100A9 on LPS-induced neutrophilia.

#### **MODES OF CARRYING OUT THE INVENTION**

In accordance with the present invention, there is provided a method and compositions for the modulation of the activity of different factors involved in the manifestations or reactions of body inflammation. The factors can cause migration of cells, such as for example but without limiting it to, neutrophils, or can cause oxidation by radicals, or proteolysis by different enzymes or proteases.

The present invention shows that myeloid-related proteins (MRP) play a role in the process of neutrophil migration to inflammatory site. MRP proteins are a subfamily of S100 proteins in which three members have been characterized, namely S100A8, S100A9, and S100A12. These small proteins are constitutively expressed at high levels in the cytosol of neutrophils. S100A8 and S100A9 are also expressed by activated endothelial cells, certain epithelial cells, keratinocytes, monocytes and activated macrophages. In the presence of calcium, S100A8 and S100A9 associate noncovalently to form the heterodimer S100A8/A9.

Several proinflammatory activities have been identified for these proteins. *In vitro* studies described herein ~~below~~ demonstrate that S100A8,

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Polyclonal antisera against human and murine recombinant S100A8 and S100A9 were generated after repeated injections in New Zealand White rabbits or CD1 rats at 4 or 2 weeks intervals respectively. Antisera titers were determined using direct ELISA and immunoblot. IgG from antisera were purified by protein A affinity chromatography (PIERCE, Rockford, Illinois)

#### Air pouch experiments

Ten to twelve weeks old CD-1 or BALB/c mice were obtained from Charles River, St-Colomban, Canada. Air pouches were raised on the dorsum by s.c. injection of 3 ml of sterile air on days 0 and 3. On day 7, 1.5 mg of MSU crystals suspended in a volume of 1 ml of endotoxin-free PBS (Sigma, St-Louis, MO) was injected into the air pouches. Alternatively, 1 ml of murine S100A8 or S100A9 at concentrations ranging from 0.01 to 10 µg/ml was injected into the air pouches. At specific times, the mice were killed by asphyxiation using CO<sub>2</sub>, the air pouches were washed once with 1 ml of PBS-5 mM EDTA, and then twice with 2 ml of PBS-5 mM EDTA, and the exudates were centrifuged at 500 x g for 5 minutes at room temperature. Cells were counted with a hemacytometer following acetic blue staining. Characterization of leukocyte subpopulations was performed by Wright-Giemsa staining of cytospin™ (VWR, Mississauga, Canada). In separate experiments, mice were injected i.p. 16 hours prior to injection of MSU crystals in the air pouch with 2 mg of purified IgG from rabbit antisera against murine S100A8 and S100A9 to inhibit their activities.

#### ELISAs

The detection of human and murine S100A8, S100A9, and S100A8/A9 was performed by coating 96-well plates with (100 µl/well) of human S100A8/A9-specific mAb 5.5 (generous gift of Nancy Hogg, IORF, London, UK), purified rabbit IgG against mouse S100A8 or mouse S100A9 (for detection of murine S100A9 and S100A8/A9), diluted to a concentration of 1